Remarks

Claims 1-16, 20-29 and 48-54 were pending in this case, of which claims 20-29 and 51-53 are withdrawn from consideration. Claims 1-16, 48-50 and 54 are subject to final rejection. Claims 1, 2, 9, 11, 12 and 48 are amended herein. Claims 55-59 are added. All of the additions and amendments are supported by the Specification and claims as originally filed; no new matter is introduced by this amendment.

After entry of this amendment, **claims 1-16, 20-29 and 48-59 are pending** in the application, of which claims 20-29 and 51-53 are currently withdrawn. It is believed that the claims are in condition for allowance, which action is requested.

Claim Rejections under 35 U.S.C. § 102(b) -- Vasa *et al.* (*Circ. Res.*)

Claims 1, 4-6, 9, 16, 48-50 and 54 are rejected under 35 U.S.C. § 102(b) as allegedly being anticipated by Vasa *et al.*, *Circ. Res.*, 89:E1-7, 2001 (hereafter "Vasa *et al.* (*Circ. Res.*)"). Applicants traverse this rejection, to the extent that it is maintained after entry of the amendments made herewith, and renew their arguments made in previous responses and incorporate those arguments herein.

Vasa et al. (Circ. Res.) does not disclose comparing a number of EPCs from a healthy subject with a number of EPCs from a healthy subject that has a low Framingham Risk Score

The method of claims 1, 4-6, 9, 16, 48-50 and 54 is not anticipated by Vasa *et al.* (*Circ. Res.*) because the claimed method requires a comparison of EPC number or function between blood samples taken from subjects without symptomatic cardiovascular disease. Vasa *et al.* (*Circ. Res.*) only disclose a comparison of EPC numbers between healthy subjects and subjects with symptomatic cardiovascular disease. However, Vasa *et al.* (*Circ. Res.*) does not disclose that healthy subjects can be stratified for the risk of developing cardiovascular disease.

Amended claims 1, 4-6, 48-50 and 54 are directed to a method of identifying a subject without symptomatic cardiovascular disease who has decreased vascular function or increased cardiovascular risk. This method requires comparing the circulating EPC numbers in a blood sample from the subject with a control, wherein the control is a number of endothelial progenitor cells in a blood sample from a control subject who does not have symptomatic cardiovascular disease and also has a low Framingham Risk Score. Further, amended claims 9 and 16 are directed to a method of diagnosing increased vascular function in a subject by detecting an increase in the number of EPCs in blood samples taken sequentially from the same subject. Support for these amendments can be found at least on page 32 lines 3-8 and page 34, line 26 to page 35, line 4 of the Specification as filed. None of these method steps are taught by Vasa *et al.* (*Circ. Res.*), as discussed below.

Vasa *et al.* (*Circ. Res.*) disclose that the number of endothelial progenitor cells (EPCs) is reduced in subjects with coronary artery disease (CAD) as compared to subjects with no evidence of CAD by history or physical exam. In addition, EPCs from subjects with CAD had an impaired migratory response. Vasa *et al.* (*Circ. Res.*) suggest that the reduced number and function of EPCs correlates with adult neovascularization. Additionally, an analysis of various risk factors showed that smoking and age correlated with reduced numbers of EPCs. However, Vasa *et al.* (*Circ. Res.*) does not describe any differences in the healthy control subjects, they are identified as simply a single unified population, and no comparison is made between healthy subjects with different Framingham risk scores. Thus, Vasa *et al.* (*Circ. Res.*) does not suggest that a decrease in the number of EPCs will affect vascular function in a healthy subject without coronary artery disease, nor does it provide methods to identify those completely healthy subjects without symptomatic cardiovascular disease who are at risk for developing the disease.

Thus, claims 1, 4-6, 9, 16, 48-50 and 54 as amended are not anticipated, nor rendered obvious, by Vasa *et al.* (*Circ. Res.*). Therefore, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Vasa et al. (Circ. Res.) do not disclose assaying a number of senescent EPCs

Additionally, amended claims 48-50 are novel over Vasa *et al.* (*Circ. Res.*) because these claims require assaying a number of senescent endothelial progenitor cells in a blood sample from a subject—a method step that Vasa *et al.* (*Circ. Res.*) do not teach. The Office action alleges that because Vasa *et al.* (Circ Res.) teach that increased age correlates with a decrease in number and migratory activity of EPCs, that this reference inherently teaches that increased EPC senescence correlates with a decrease in number and migratory activity of EPCs. This is not correct.

Applicants respectfully submit that the age of a subject is not synonymous with the number of senescent EPCs in blood samples taken from that subject. Erusalimsky and Kurz (Handbook of Experimental Pharmacology 176: 213-248, 2006, abstract attached) teach that endothelial cell senescence can be induced by genetic discorders, teleomere damage, oxidative stress and sustained mitogenic stimulation, as well as age. Eursalimky and Kurz indicate that there is data that "argues for and against a role of endothelial cell senescence in age-related vascular pathology." Thus, it cannot be inferred that age will be simply correlated with the number of senescent EPCs, especially if the subjects are exposed to oxidative stress (such as smoking).

Thus, as Vasa *et al.* (*Circ. Res.*) does not teach assaying the number of senescent endothelial progenitor cells, this reference does not anticipate, nor render obvious, claims 48-50.

Vasa et al. (Circ. Res.) do not disclose diagnosing increased vascular function in a subject

As amended, claims 9 and 16 are directed to a method of diagnosing increased vascular function in a subject by detecting an increase in the number of EPCs in blood samples taken sequentially *from the same subject*. For example, claim 9 requires, *inter alia*, "assaying a number of endothelial progenitor cells in first and second blood samples taken from the subject, wherein the second blood sample is taken from the subject after the first blood sample is taken from the subject...wherein an increase in the number of endothelial progenitor cells in the second blood sample as compared to the[[a]] first blood sample indicates increased vascular function." However, Vasa *et al.*

(*Circ. Res.*) teach a method of comparing circulating EPCs between a subject with symptomatic cardiovascular disease and a separate control group of subjects that lack symptomatic cardiovascular disease. Thus, because this reference does not teach each of the steps of the claimed method, expressly or inherently, Applicants submit anticipate the method of claims 9 and 16 is novel over Vasa *et al.* (*Circ. Res.*) and respectfully request withdrawal of the instant rejection.

Claim Rejections under 35 U.S.C. § 102(b) -- Vasa et al. (Circulation)

Claims 9-11 and 16 are rejected under 35 U.S.C. § 102(b) as allegedly anticipated by Vasa *et al.*, *Circulation*, 103:2885-2890, 2001 (hereafter "Vasa *et al.* (*Circulation*)"). Applicants traverse this rejection, to the extent that it is maintained after entry of the amendments made herewith, and renew their arguments made in previous responses and incorporate those arguments herein.

As amended, claim 9 is directed to a method of identifying a subject that does not have symptomatic cardiovascular disease, but does have increased vascular function, by comparing the circulating EPC numbers in a first blood sample from the subject with a second blood sample taken from the same subject. An increase in the number of endothelial progenitor cells in the second blood sample as compared to the first blood sample indicates increased vascular function. Claims 10, 11 and 16 are directed to limitations of claim 9, wherein the subject has been treated with a cholesterol lowering agent or the control is a blood sample from such a subject or wherein assaying the number of EPCs comprises determining the number of $VEGFR^{2+}CD31^{hi}$ cells in the sample.

Vasa *et al.* (*Circulation*) describe that statin treatment of patients with symptomatic cardiovascular disease is associated with an increase in the number of circulating endothelial progenitor cells, as well as an increase in the migratory capacity in response to vascular endothelial growth factor of endothelial progenitor cells. Additionally, this reference discloses that healthy subjects treated with Atorvastatin experienced an increase in circulating EPC numbers compared to healthy control subjects. Thus, Vasa *et al.* (*Circulation*) suggest that the increase in the number and migratory activity of EPCs resulting from statin treatment contributes to the angiogenesis and vasculogenesis that lead to neovascularization. The Office action asserts that because an increase in

the number of endothelial progenitor cells is associated with statin therapy and that statin therapy is associated with increased vascular function, that an increase in the number of endothelial progenitor cells must be associated with improved vascular function (and thus decreased risk for cardiovascular disease), even in healthy subjects.

However, Vasa *et al.* (*Circulation*) do not teach that an increase in EPC number *in healthy subjects* is associated with any kind of increased vascular function. Further, the teachings of this reference are directed towards the treatment of subjects with cardiovascular disease with Atorvastatin, and not directed to the claimed method of diagnosing subjects with increased vascular function. Vasa *et al.* (*Circulation*) does teach that there is an increased number of circulating EPCs in healthy subjects treated with Atorvastatin, but does not suggest, nor render obvious, that this increase is associated with increased vascular function *in healthy subjects,* and thus reduced risk for developing cardiovascular disease in the future. Thus, one of skill in the art, reading Vasa *et al.* (*Circulation*), would not glean the method of claims 9 and 16, expressly or inherently. Therefore, Applicants respectfully request reconsideration and withdrawal of the instant rejection.

Claim Rejections under 35 U.S.C. § 112, Second Paragraph

Claims 2-3, 7-8, 12-15 and 48-50 are rejected under 35 U.S.C. § 112, second paragraph, as allegedly being indefinite for not clearly defining the steps in the method. Applicants traverse this rejection, to the extent that it is maintained after entry of the amendments made herewith, and renew their arguments made in previous responses and incorporate those arguments herein.

Claims 2 and 12 are rejected under 35 U.S.C. § 112, second paragraph as allegedly the steps of the method are unclear. Applicants respectfully disagree with the rejection. However, to advance prosecution, claims 2 and 12 are amended to include the phrase "confirming that the subset of the non-adherent cells that adhered to the second substrate are endothelial progenitor cells by immunological assessment." Support for these amendments can be found throughout the specification, and at least on page 28, line 23 to page 29, line 2 of the Specification as filed. As amended, claims 2 and 12 define specific methods of assaying for endothelial progenitor cells. Thus, the undersigned believes the

amendments clarify the steps of the method, and render the rejection moot. If additional amendments are required, the Examiner is respectfully requested to telephone the undersigned for an interview. Claims 56-57 are added herein, which further limit the method of claims 2 and 12, respectively.

Claims 48-50 are rejected under 35 U.S.C. § 112, second paragraph as allegedly the "senescent" endothelial progenitor cells are indefinite, as there are not specific phenotypic features that delineate a senescent cell in view of a non-senescent cell. Applicants respectfully disagree with this rejection, as the characteristics of a senescent endothelial cell are known to one of skill in the art. However, solely to advance prosecution, claim 48 is amended to clarify that the senescent cell "is a viable endothelial cell that exhibits clonal exhaustion *in vitro.*" Additionally, claim 58 is added, which defines a further step in the method, namely evaluating the expression of endogenous beta-galactosidase. Support for these amendments can be found at least on page 34, line 20 to page 35, line 4 of the Specification as filed.

Reconsideration and withdrawal of the rejection are respectfully requested.

Conclusion

Applicants believe the present application is ready for allowance, which action is requested. If any matters remain to be discussed before a Notice of Allowance is issued, Examiner Kaushal is respectfully requested to contact the undersigned for a telephone interview at the telephone number listed below. This request is being submitted under MPEP § 713.01, which indicates that an interview may be arranged in advance by a written request.

Respectfully submitted,

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Résumé / Abstract
The wear and tear processes that are thought to contribute to human ageing may play an important role in the development of vascular diseases. One such process is cellular senescence. In endothelial cells the senescent phenotype can be induced by a number of factors, including telomere damage, oxidative stress and sustained mitogenic stimulation. Several lines of evidence indicate that endothelial cell senescence may be relevant to vascular disease. In this chapter we examine the causes, mechanisms and regulation of endothelial cell senescence as they emerge from studies in cell culture. We also describe the senescent phenotype and discuss its pathophysiological implications. We review the evidence for the occurrence of endothelial cell senescence in vivo and examine findings in animal models of ageing and human genetic disorders that argue for and against a role of endothelial cell senescence in age-related vascular pathology. Finally, we address the particular case of endothelial progenitor cell senescence and discuss the relevance of this phenomenon for angiogenesis and vascular repair.

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